Remarks/Arguments

Claims 44 - 46, 49 and 52 - 60 are pending in this application.

Amendments

Claims 44-46, 49, 52-60 are currently pending. Claims 44-46, 49, 57 and 58 are allowed.

Claims 52-56 have been amended to recite that the polypeptide inhibits VEGF stimulated proliferation of endothelial cells. Support for this amendment can be found in the specification at, for example, Example 66, page 204. Applicants expressly reserve the right to pursue any canceled matter in subsequent continuation, divisional or continuation-in-part applications.

Information Disclosure Statement

Applicants thank the Examiner for consideration of the Information Disclosure Statement filed on December 8, 2004 and partial consideration of the Information Disclosure Statement filed on November 18, 2004.

Priority

Based on the ability of the claimed polypeptides to inhibit VEGF stimulated proliferation of adrenal cortical capillary endothelial cells, which was disclosed in application PCT/US00/04414, the Examiner accorded February 22, 2000 as the earliest priority date to the present application. However, Applicants believe that they should be accorded the priority date of **September 16, 1998** which is the filing date of PCT Application No. PCT/US98/19330. Applicants enclose herewith pages 54 and170-172 (Example 66) and Figure 2 from that PCT/US98/19330 which shows that PRO211 inhibited VEGF stimulated proliferation of endothelial cells.

35 U.S.C. §112, First Paragraph, Rejections

1. Claims 52-56 and 59-60 stand rejected under 35 U.S.C. §112, first paragraph, for lack of enablement allegedly because a polypeptide having at least 80% amino acid sequence identity to the polypeptide of SEQ ID NO:2 which isolated polypeptide inhibits VEGF stimulated proliferation of adrenal cortical capillary endothelial cells, does not reasonably provide enablement for a polypeptide not identical to at least the mature form of SEQ ID NO:2 which does not have this activity.

Applicants have amended claims 52-56 to recite that the polypeptides inhibit VEGF stimulated proliferation of endothelial cells. Applicants claim priority to PCT/US98/19330, filed September 16, 1998.

The Examiner has previously stated that the specification is enabling for the full length polypeptide of SEQ ID NO:2 which inhibits VEGF stimulated proliferation of adrenal cortical endothelial cells.

Further Applicants submit that the amended claims 52-56 are not directed to a genus of polypeptides of any function, but rather, to polypeptides that have a specific and substantially useful function, i.e. to polypeptides that inhibit VEGF stimulated proliferation of endothelial cells.

The Legal Test for Enablement

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosure provided by applicants coupled with information known in the art at the time the invention was made, without undue experimentation.[1] [2]. Accordingly, the test for enablement is not whether any experimentation is necessary, but whether, if experimentation is required, it is undue.[3] The mere fact that an

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[1] MPEP §2164.0120

[2] *United States v. Telectronics, Inc.* 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1998)) *United States v. Telectronics, Inc.* 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1998))

[3] *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976)

extended period of experimentation is necessary does not make such experimentation undue.[4] [5]

A finding of lack of enablement and a determination that undue experimentation is necessary requires an analysis of a variety of factors *(i.e.,* the *In re* Wands factors). The most important factors that must be considered in this case include 1) the nature of the invention; 2) the level of one of ordinary skill in the art; 3) guidance provided in the specification, 4) the state of the prior art, and 8) the breadth of the claims.

"How a teaching is set forth, by specific example or broad terminology, is not important"[6] [7]. "Limitations and examples in the specification do not generally limit what is covered by the claims" MPEP § 2164.08. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. It is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art. The legal standard merely requires that there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and use the invention as broadly as it is claimed.[8]

The Disclosure provides sufficient information to enable the claimed invention

Claims 52-56 are directed to a genus of polypeptides which are at least 80-99% identical to SEQ ID NO:2 and which have a specific and useful function (*i.e.* to a genus of polypeptides which inhibit VEGF stimulated proliferation of endothelial cells).

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[4] *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977)

[5] MPEP §2164.06.

[6] MPEP §2164.08

[7] *In re Marzocchi*, 439 F. 2d 220, 223-4, 169 USPQ 367, 370 (CCPA 1971)

[8] *Enzo Biochem., Inc. v. Calgene, Inc.*, 188 F.3d 13 62 (Fed. Circ. 1999), at 1372 (quoting *In re* Vaeck, 947 F.2d 488, 496 (Fed. Cir. 1991)).

Applicants have provided native PRO sequence SEQ ID NO:2. The present application describes methods for identifying peptides which inhibit VEGF stimulated proliferation of endothelial cells. Example 66 of the present application provides step-by-step guidelines and protocols for the VEGF proliferation assay. The specification further describes methods for the determination of percent identity between two amino acid sequences. (pages 67 - 69). In fact, the specification teaches specific parameters to be associated with the term "percent identity" as applied to the present invention. Accordingly, one of skill in the art could identify whether the variant PRO211 native sequence falls within the parameters of the claimed invention. Once such an amino acid sequence was identified, the specifications sets forth methods for making the amino acid sequences (pages 112-115) and methods of preparing the PRO polypeptides (pages 117-122). Accordingly, one skilled in the art given the disclosure in the specification would be able to make the claimed amino acid sequence. Furthermore, one of ordinary skill in the art has a sufficiently high level of technical competence to identify sequences with at least 80% identity to SEQ ID NO:2. Accordingly, one of ordinary skill could practice the claimed invention without undue experimentation.

The Examiner objects to the Declaration of Goddard, Avi Ashkenazi and Polakis. The Examiner presents articles by Pennica et al., and Konopka et al. regarding the alleged lack of correlation between gene amplification and increased polypeptide levels. The Examiner also presents an article by Hu et al., regarding an alleged lack of correlation between gene expression levels and a known role in cancer.

Applicants do not agree with the arguments presented by the Examiner. However, Applicants have amended claims 52-56 to recite that the polypeptide inhibits VEGF stimulated proliferation of endothelial cells. Accordingly, the claims no longer recite that the polypeptide is associated with colon or lung tumor. Accordingly, these objections are no longer applicable and the objections are rendered moot.

For the reasons set forth above, the Examiner is respectfully requested to withdraw the rejection under 35 U.S.C. 112, first paragraph for lack of enablement.

2. Claims 52-56 and 59-60 stand rejected under 35 U.S.C. §112, first paragraph, for lack of written description for the reasons set forth in Section 8 of paper mailed on October 2, 2002 and in section 6 of Paper mailed September 3, 2003. In Section 6 of Paper mailed September 3, 2003, the Office action stated that

"the claims, as amended, recite the activity of the polypeptides as "associated with the formation or growth of lung or colon tumor" and this asserted activity clearly lacks support in the instant specification, as filed, see reasons of record in section 5 earlier in the instant office action. Thus because the claims are directed to polypeptides having 80%, 85%, 90% 95% or 99% sequence identity which a particular disclosed sequence and require the polypeptides to possess the activity, for which no functional assay is disclosed, the claims are directed to subject matter which was not described in the specification"

Applicants have amended Claims 52-56 to recite that the polypeptides inhibit VEGF stimulated proliferation of endothelial cells. As discussed above, the specification in Example 66 provides guidance for identifying polypeptides with this functional activity. Accordingly, this rejection is rendered moot. Withdrawal of this rejection is respectfully requested.

The present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited.

CONCLUSION

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (Attorney's Docket No. **39780-1618 P2C5**). Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

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SV 2127604 v1
5/27/05 3:44 PM(39780.1618)

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, 15/18, 15/52, C07K 14/47, 14/705, C12N 15/62, C07K 16/18, 16/28

A2

(11) International Publication Number: WO 99/14328

(43) International Publication Date: 25 March 1999 (25.03.99)

(21) International Application Number: PCT/US98/19330

(22) International Filing Date: 16 September 1998 (16.09.98)

(30) Priority Data:

60/059,115	17 September 1997 (17.09.97)	US
60/059,184	17 September 1997 (17.09.97)	US
60/059,122	17 September 1997 (17.09.97)	US
60/059,117	17 September 1997 (17.09.97)	US
60/059,113	17 September 1997 (17.09.97)	US
60/059,121	17 September 1997 (17.09.97)	US
60/059,119	17 September 1997 (17.09.97)	US
60/059,263	18 September 1997 (18.09.97)	US
60/059,266	18 September 1997 (18.09.97)	US
60/062,125	15 October 1997 (15.10.97)	US
60/062,287	17 October 1997 (17.10.97)	US
60/062,285	17 October 1997 (17.10.97)	US
60/063,486	21 October 1997 (21.10.97)	US
60/062,816	24 October 1997 (24.10.97)	US
60/062,814	24 October 1997 (24.10.97)	US
60/063,127	24 October 1997 (24.10.97)	US
60/063,120	24 October 1997 (24.10.97)	US
60/063,121	24 October 1997 (24.10.97)	US
60/063,045	24 October 1997 (24.10.97)	US
60/063,128	24 October 1997 (24.10.97)	US
60/063,329	27 October 1997 (27.10.97)	US
60/063,327	27 October 1997 (27.10.97)	US
60/063,549	28 October 1997 (28.10.97)	US
60/063,541	28 October 1997 (28.10.97)	US
60/063,550	28 October 1997 (28.10.97)	US
60/063,542	28 October 1997 (28.10.97)	US
60/063,544	28 October 1997 (28.10.97)	US
60/063,564	28 October 1997 (28.10.97)	US
60/063,734	29 October 1997 (29.10.97)	US
60/063,738	29 October 1997 (29.10.97)	US
60/063,704	29 October 1997 (29.10.97)	US
60/063,435	29 October 1997 (29.10.97)	US
60/064,215	29 October 1997 (29.10.97)	US
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60/064,103	31 October 1997 (31.10.97)	US
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60/064,248	3 November 1997 (03.11.97)	US
60/064,809	7 November 1997 (07.11.97)	US
60/065,186	12 November 1997 (12.11.97)	US
60/065,846	17 November 1997 (17.11.97)	US
60/065,693	18 November 1997 (18.11.97)	US
60/066,120	21 November 1997 (21.11.97)	US
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60/066,772	24 November 1997 (24.11.97)	US
60/066,466	24 November 1997 (24.11.97)	US
60/066,770	24 November 1997 (24.11.97)	US
60/066,511	24 November 1997 (24.11.97)	US
60/066,453	24 November 1997 (24.11.97)	US
60/066,840	25 November 1997 (25.11.97)	US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME

(57) Abstract

The present invention is directed to novel polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptides molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding PRO244 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding PRO244 polypeptide having amino acid residues 1 to 219 of Fig. 122 (SEQ ID NO:377), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions.

In another embodiment, the invention provides isolated PRO244 polypeptide. In particular, the invention provides isolated native sequence PRO244 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 219 of Figure 122 (SEQ ID NO:377).

50. Additional Embodiments

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the above or below described polypeptides. A host cell comprising any such vector is also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the above or below described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the above or below described polypeptides fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises any of the above or below described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences, wherein those probes may be derived from any of the above or below described nucleotide sequences.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a nucleotide sequence (SEQ ID NO:1) of a native sequence PRO211 cDNA, wherein SEQ ID NO:1 is a clone designated herein as "UNQ185" and/or "DNA32292-1131".

Figure 2 shows the amino acid sequence (SEQ ID NO:2) derived from the coding sequence of SEQ ID NO:1 shown in Figure 1.

Figure 3 shows a nucleotide sequence (SEQ ID NO:3) of a native sequence PRO217 cDNA, wherein SEQ ID NO:3 is a clone designated herein as "UNQ191" and/or "DNA33094-1131".

Figure 4 shows the amino acid sequence (SEQ ID NO:4) derived from the coding sequence of SEQ ID NO:3 shown in Figure 3.

Figure 5 shows a nucleotide sequence (SEQ ID NO:11) of a native sequence PRO230 cDNA, wherein SEQ ID NO:11 is a clone designated herein as "UNQ204" and/or "DNA33223-1136".

Figure 6 shows the amino acid sequence (SEQ ID NO:12) derived from the coding sequence of SEQ ID NO:11 shown in Figure 5.

Figure 7 shows a nucleotide sequence designated herein as DNA20088 (SEQ ID NO:13).

EXAMPLE 64: Determination of PRO317-Induced Cellular Response

The biological activity of PRO317 is measured, for example, by binding of an PRO317 of the invention to an PRO317 receptor. A test compound is screened as an antagonist for its ability to block binding of PRO317 to the receptor. A test compound is screened as an agonist of the PRO317 for its ability to bind an PRO317 receptor and influence the same physiological events as PRO317 using, for example, the KIRA-ELISA assay described by Sadick *et al.*, Analytical Biochemistry, 235:207-214 (1996) in which activation of a receptor tyrosine kinase is monitored by immuno-capture of the activated receptor and quantitation of the level of ligand-induced phosphorylation. The assay may be adapted to monitor PRO317-induced receptor activation through the use of an PRO317 receptor-specific antibody to capture the activated receptor. These techniques are also applicable to other PRO polypeptides described herein.

EXAMPLE 65: Use of PRO224 for Screening Compounds

PRO224 is expressed in a cell stripped of membrane proteins and capable of expressing PRO224. Low density lipoproteins having a detectable label are added to the cells and incubated for a sufficient time for endocytosis. The cells are washed. The cells are then analysed for label bound to the membrane and within the cell after cell lysis. Detection of the low density lipoproteins within the cell determines that PRO224 is within the family of low density lipoprotein receptor proteins. Members found within this family are then used for screening compounds which affect these receptors, and particularly the uptake of cholesterol via these receptors.

EXAMPLE 66: Ability of PRO Polypeptides to Inhibit Vascular Endothelial Growth Factor (VEGF) Stimulated Proliferation of Endothelial Cell Growth

The ability of various PRO polypeptides to inhibit VEGF stimulated proliferation of endothelial cells was tested. Specifically, bovine adrenal cortical capillary endothelial (ACE) cells (from primary culture, maximum 12-14 passages) were plated on 96-well microtiter plates (Amersham Life Science) at a density of 500 cells/well per 100 μL in low glucose DMEM, 10% calf serum, 2 mM glutamine, 1x pen/strept and fungizone, supplemented with 3 ng/mL VEGF. Controls were plated the same way but some did not include VEGF. A test sample of the PRO polypeptide of interest was added in a 100 μl volume for a 200 μl final volume. Cells were incubated for 6-7 days at 37°C. The media was aspirated and the cells washed 1x with PBS. An acid phosphatase reaction mixture (100 μL, 0.1M sodium acetate, pH 5.5, 0.1% Triton-100, 10 mM p-nitrophenyl phosphate) was added. After incubation for 2 hours at 37°C, the reaction was stopped by addition of 10 μl 1N NaOH. OD was measured on microtiter plate reader at 405 nm. Controls were no cells, cells alone, cells + FGF (5 ng/mL), cells + VEGF (3 ng/mL), cells + VEGF (3 ng/ml) + TGF-β (1 ng/ml), and cells + VEGF (3ng/mL) + LIF (5 ng/mL). (TGF-β at a 1 ng/ml concentration is known to block 70-90% of VEGF stimulated cell proliferation.)

The results were assessed by calculating the percentage inhibition of VEGF (3 ng/ml) stimulated cells proliferation, determined by measuring acid phosphatase activity at OD405 nm, (1) relative to cells without stimulation, and (2) relative to the reference TGF-β inhibition of VEGF stimulated activity. The results, as shown in Table 2 below, are indicative of the utility of the PRO polypeptides in cancer therapy and specifically in inhibiting tumor angiogenesis. The numerical values (relative inhibition) shown in Table 2 are determined by calculating the

percent inhibition of VEGF stimulated proliferation by the PRO polypeptide relative to cells without stimulation and then dividing that percentage into the percent inhibition obtained by TGF-β at 1 ng/ml which is known to block 70-90% of VEGF stimulated cell proliferation.

Table 2

PRO Name	PRO Concentration	Relative Inhibition
PRO211	0.01%	99.0
PRO211	0.01%	1.09
PRO211	0.1%	0.95
PRO211	0.1%	67.0
PRO211	1.0%	0.27
PRO211	1.0%	20.0
PRO217	0.01%	1.06
PRO217	0.1%	0.84
PRO217	1.0%	0.39
PRO217	2.5 μM	0.2
PRO217	25 nM	0.88
PRO217	250 nM	0.58
PRO187	0.01%	0.91
PRO187	0.1%	0.82
PRO187	1.0%	0.44
PRO219	5.7 μM	0.61
PRO219	57 nM	1.09
PRO219	570 nM	0.97
PRO246	0.01%	1.04
PRO246	0.1%	1.0
PRO246	1.0%	0.49
PRO228	0.01%	0.99
PRO228	0.1%	0.93
PRO228	1.0%	0.57
PRO228	0.01%	0.95
PRO228	0.01%	0.98
PRO228	0.1%	0.77
PRO228	0.1%	0.88
PRO228	1.0%	0.16
PRO228	1.0%	0.48
PRO245	0.01%	0.76
PRO245	0.1%	0.35
PRO245	1.0%	0.11
PRO245	0.48 nM	1.03
PRO245	4.8 nM	0.95
PRO245	48 nM	0.49
PRO221	0.01%	1.03
PRO221	0.01%	1.06
PRO221	0.1%	0.82
PRO221	0.1%	0.93
PRO221	1.0%	0.31
PRO221	1.0%	0.43
PRO258	0.01%	0.98
PRO258	0.01%	1.06
PRO258	0.1%	0.95
PRO258	0.1%	1.02
PRO258	1.0%	0.6
PRO258	1.0%	0.69

Table 2 cont'

PRO Name	PRO Concentration	Relative Inhibition
PRO301	7.0 μM	1.02
PRO301	70 μM	0.88
PRO301	700 μM	0.44
PRO301	0.01%	0.92
PRO301	0.1%	0.85
PRO301	1.0%	0.68
PRO224	0.01%	101.0
PRO224	0.1%	65.0
PRO224	1.0%	23.0
PRO272	0.01%	0.95
PRO272	0.1%	0.57
PRO272	1.0%	0.18
PRO328	0.01%	0.98
PRO328	0.1%	0.96
PRO328	1.0%	0.6
PRO331	0.01%	0.88
PRO331	0.1%	0.82
PRO331	1.0%	0.56

EXAMPLE 67: Retinal Neuron Survival

This example demonstrates that PRO220 polypeptides have efficacy in enhancing the survival of retinal neuron cells.

Sprague Dawley rat pups at postnatal day 7 (mixed population: glia and retinal neuronal types) are killed by decapitation following CO_2 anesthesia and the eyes are removed under sterile conditions. The neural retina is dissected away from the pigment epithelium and other ocular tissue and then dissociated into a single cell suspension using 0.25% trypsin in Ca^{2+}, Mg^{2+}-free PBS. The retinas are incubated at 37°C for 7-10 minutes after which the trypsin is inactivated by adding 1 ml soybean trypsin inhibitor. The cells are plated at 100,000 cells per well in 96 well plates in DMEM/F12 supplemented with N2 and with or without the specific test PRO polypeptide. Cells for all experiments are grown at 37°C in a water saturated atmosphere of 5% CO_2. After 2-3 days in culture, cells are stained with calcein AM then fixed using 4% paraformaldehyde and stained with DAPI for determination of total cell count. The total cells (fluorescent) are quantified at 20X objective magnification using CCD camera and NIH image software for MacIntosh. Fields in the well are chosen at random.

The effect of various concentration of PRO220 polypeptides are reported in Table 3 below where percent survival is calculated by dividing the total number of calcein AM positive cells at 2-3 days in culture by the total number of DAPI-labeled cells at 2-3 days in culture. Anything above 30% survival is considered positive.

Table 3

PRO Name	PRO Concentration	Percent Survival
PRO220	0.01%	2.4%
PRO220	0.01%	4.1%
PRO220	0.1%	3.0%
PRO220	0.1%	3.1%
PRO220	1.0%	72.4%
PRO220	1.0%	42.1%

FIGURE 2

Met Arg Leu Pro Arg Arg Ala Ala Leu Gly Leu Leu Pro Leu Leu Leu
Leu Leu Pro Pro Ala Pro Glu Ala Ala Lys Lys Pro Thr Pro Cys His
Arg Cys Arg Gly Leu Val Asp Lys Phe Asn Gln Gly Met Val Asp Thr
Ala Lys Lys Asn Phe Gly Gly Gly Asn Thr Ala Trp Glu Glu Lys Thr
Leu Ser Lys Tyr Glu Ser Ser Glu Ile Arg Leu Leu Glu Ile Leu Glu
Gly Leu Cys Glu Ser Ser Asp Phe Glu Cys Asn Gln Met Leu Glu Ala
Gln Glu Glu His Leu Glu Ala Trp Trp Leu Gln Leu Lys Ser Glu Tyr
Pro Asp Leu Phe Glu Trp Phe Cys Val Lys Thr Leu Lys Val Cys Cys
Ser Pro Gly Thr Tyr Gly Pro Asp Cys Leu Ala Cys Gln Gly Gly Ser
Gln Arg Pro Cys Ser Gly Asn Gly His Cys Ser Gly Asp Gly Ser Arg
Gln Gly Asp Gly Ser Cys Arg Cys His Met Gly Tyr Gln Gly Pro Leu
Cys Thr Asp Cys Met Asp Gly Tyr Phe Ser Ser Leu Arg Asn Glu Thr
His Ser Ile Cys Thr Ala Cys Asp Glu Ser Cys Lys Thr Cys Ser Gly
Leu Thr Asn Arg Asp Cys Gly Glu Cys Glu Val Gly Trp Val Leu Asp
Glu Gly Ala Cys Val Asp Val Asp Glu Cys Ala Ala Glu Pro Pro Pro
Cys Ser Ala Ala Gln Phe Cys Lys Asn Ala Asn Gly Ser Tyr Thr Cys
Glu Glu Cys Asp Ser Ser Cys Val Gly Cys Thr Gly Glu Gly Pro Gly
Asn Cys Lys Glu Cys Ile Ser Gly Tyr Ala Arg Glu His Gly Gln Cys
Ala Asp Val Asp Glu Cys Ser Leu Ala Glu Lys Thr Cys Val Arg Lys
Asn Glu Asn Cys Tyr Asn Thr Pro Gly Ser Tyr Val Cys Val Cys Pro
Asp Gly Phe Glu Glu Thr Glu Asp Ala Cys Val Pro Pro Ala Glu Ala
Glu Ala Thr Glu Gly Glu Ser Pro Thr Gln Leu Pro Ser Arg Glu Asp
Leu